

Review Article

<https://doi.org/10.20546/ijcmas.2026.1506.007>

Recent Advances in Canine Blood Transfusion: A Review

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Keywords

Canine transfusion,
blood components,
blood typing,
crossmatching,
platelet-rich plasma,
transfusion reactions,
veterinary medicine

Article Info

Received:
14 April 2026
Accepted:
26 May 2026
Available Online:
10 June 2026

ABSTRACT

Transfusion medicine is a vital component of emergency and critical care management of canine patients. Whole blood transfusion has been remained as a life saving measure in both human and animal health practices. However, advances in component therapy have replaced traditional whole blood transfusion, allowing targeted treatment of specific deficiencies such as anemia, coagulopathies, and thrombocytopenia. In dogs, blood group systems are defined by specific red blood cell antigens, making blood typing and compatibility testing essential to prevent alloimmunization and adverse reactions. Understanding these aspects is essential for ensuring safe and effective transfusion practices in veterinary medicine especially in canine medicine.

Introduction

Transfusion medicine is a multidisciplinary science

concerned with the proper use of blood or blood products in the treatment or prevention of disease (Wardrop, 2008).

Transfusion is an intravenous infusion of blood or its components that have an important role in life-saving and advanced treatment of critically ill patients (Khan and Sharma, 2021, Ramakant *et al.*, 2023). In transfusion medicine, blood transfusion appears to be the most important lifesaving modality in small animal practice (Ramakant *et al.*, 2023). In blood transfusion, whole blood transfusion has been replaced with more specialized component transfusion, due to advancement in canine medicine (Davidow, 2013). Component transfusion, being a goal directed therapy, aims to replace the specific component required by recipient. Component therapy allows for efficient use of blood resources and decreases risk of transfusion reactions by delivering only the blood component(s) needed by a recipient (Yagi and Spromberg, 2018).

History of transfusion medicine

History begins with the discovery of William Harvey's theory of circulation in 1628 which made possibilities for advancement in this area. First Successful Canine Blood Transfusion was conducted by an English physician Richard Lower in 1665 (Davidow, 2013, Aravindh & Jacob, 2021). Karl Lansteiner discovered blood groups in 1900 and emphasized the importance of cross matching prior to transfusion (Choudhary *et al.*, 2017). In 1914, Hustin used Citrate as an anticoagulant for the first time and transfused blood safely in dogs (Ramakant *et al.*, 2023). The amount of citrate to be transfused safely in dogs as an effective anticoagulant was determined by Richard Lewisohn (Choudhary *et al.*, 2017). In the 1960s, Pool and co-workers discovered that when frozen plasma was allowed to thaw slowly, it precipitated and became a rich source of FVIII, vWF, XIII, and fibrinogen (Wong and Curry, 2016).

Blood transfusion

Indications

Transfusion therapies are indicated for the treatment of anemia, coagulopathies, thrombocytopenia and hypoproteinemia (Dadke and Galdhar, 2021, Ramakant *et al.*, 2023). Blood transfusion can either be whole blood transfusion or component transfusion.

Whole blood transfusion

In the whole blood transfusion, whole blood is pulled

from a donor without any processing. It can be stored for 28 days in CPDA-1 at 1–6°C, principally indicated for the treatment of anemia and substantial blood loss (Davidow, 2013, Ramakant *et al.*, 2023). Exact volume of blood to be transfused can be calculated by the following formula:

$$\text{Volume of blood to be transfused (ml)} = \text{Body Weight (kg)} * k * \left(\frac{\text{Hct}_{\text{recipient}} - \text{Hct}_{\text{donor}}}{\text{Hct}_{\text{donor}}} \right) * 100$$

k = Volume of blood (ml/kg) in dogs it is 80-90 ml/kg

For the initial 15 minutes rate of infusion should be 0.5-1 ml/kg/hour followed by 5-10 ml/kg/hour and transfusion should be completed within 4 hours (Aravindh and Jacob, 2021).

Component transfusion

The whole blood is separated into various components, when whole blood is subjected to light spin it gets separated into RBCs and platelets along with plasma, platelets along with plasma undergoes heavy spin to yield platelet rich plasma and platelet poor plasma, platelet poor plasma is frozen and termed as fresh frozen plasma. When fresh frozen plasma is allowed to thaw slowly, it gives cryoprecipitate and cryoprecipitate poor plasma (Liu *et al.*, 2019, Hausauer, 2021).

Applications of PRP

Blood groups of canines

Canine blood group system is defined by the expression of various antigens on the surface of RBCs. the acronym DEA is used now for "Dog Erythrocyte Antigen", followed by the numerical designation of the blood group classified with polyclonal alloantibodies. Six DEA types have been recognized internationally: DEA 1, 3, 4, 5, 7 and 8. Within the DEA 1 system, three antigens (1, 2, and 3, or 1.1, 1.2, and 1.3) have been described. In general, the prevalence of DEA 1 in the canine population is about 60% (Esteves *et al.*, 2011). But in recent studies using a newer monoclonal DEA 1 antibody describes the DEA 1 antigen as an autosomal dominant inheritance pattern with 4-5 possible alleles. This means that, rather than being positive or negative for DEA 1.1, 1.2, or 1.3, dogs are either positive or negative for DEA 1, but the strength of that "positivity" depends on which

form of the gene they have inherited. By this system, dogs are either negative for DEA 1 or positive ranging from 1+ to 4+ (Zaremba *et al.*, 2019). In 2007, the Dal blood group was identified in an anemic Dalmatian dog through the use of a gel agglutination assay. The Dal blood group is characterized by anti-*Dal* alloantibodies; Dal is an antigen in red cells associated with anti-Dal alloantibody production (Blais *et al.*, 2007). Another blood group involves the Kai systems developed by mouse hybridoma techniques. Kai was studied in South Korea via the use of monoclonal antibodies, anti-Kai 1, and anti-Kai 2 by Lee and co-workers in 2017 (Lee *et al.*, 2017).

Blood Typing

Blood typing is an essential procedure in veterinary transfusion medicine to prevent the development of alloantibodies against red blood cells (RBCs) and to reduce the risk of transfusion reactions. Various methods have been developed for canine blood typing, including the Michigan State University (MSU) tube test, card agglutination test, gel column assay, flow cytometry, automated cartridge systems, and immunochromatographic strip tests.

All these techniques are based on the principle of antigen-antibody agglutination between RBC surface antigens and specific monoclonal or polyclonal antisera (Weinstein and Sink, 2012).

Michigan State University (MSU) Test

The MSU test is a tube agglutination assay enhanced by the addition of canine Coombs' reagent and is primarily used for DEA 1 typing. Washed RBC suspensions are incubated with polyclonal antisera against different DEA antigens (DEA 1.X, 1.1, 3, 4, and 5), followed by centrifugation and assessment of agglutination strength. Agglutination reactions are graded from 0 to 4+, with reactions $\geq 2+$ considered positive. Coombs' reagent is used to enhance weak agglutination reactions.

Card Agglutination Method

The card agglutination test is a rapid in-clinic method that uses whole blood and monoclonal antibodies immobilized on card wells. The card typically contains wells for autoagglutination control, positive control, and patient testing.

After mixing EDTA blood with diluent and reagents, the card is gently rocked and examined for agglutination. Any visible agglutination indicates a positive result for the respective blood group (Ebelt *et al.*, 2020).

Gel Column Method

The gel column assay detects agglutination reactions within microcolumns prefilled with dextran-acrylamide gel. A diluted RBC suspension and alloantibody reagent are added to the gel column and centrifuged. Agglutinated RBCs remain trapped in the gel matrix, while non-agglutinated cells settle at the bottom. Results are graded from negative to 4+ depending on the position of RBCs within the column (Ebelt *et al.*, 2020).

Flow Cytometry

Flow cytometry is a sensitive technique that detects RBC antigens using fluorescently labeled antibodies. Cells passing through a laser beam generate signals based on light scattering and fluorescence emission, which correlate with cell size, complexity, and antigen-antibody binding.

This method allows accurate detection of RBC surface antigens and is useful for detailed immunophenotyping in research and advanced diagnostic laboratories (Adan *et al.*, 2017; Santos *et al.*, 2020).

Automated Cartridge Typing System

Automated cartridge-based analyzers are designed for rapid DEA 1.1 typing. The system uses disposable cartridges with capillary channels where diluted blood samples flow by capillary action. Agglutination is detected by measuring changes in transmitted light intensity, and results are automatically reported as positive, negative, or inconclusive within a few minutes (Kohn *et al.*, 2012).

Immunochromatographic Strip Test

The immunochromatographic strip test is a lateral flow assay that uses monoclonal anti-DEA 1 antibodies immobilized on a nitrocellulose membrane. When a blood sample mixed with buffer migrates along the strip, RBCs expressing the antigen bind to the antibody at the test line, producing a visible red band. The intensity of the band is semi-quantitatively graded from negative to

4+ (Bahadir and Sezgintürk, 2016; Ebel et al., 2020).

Selection of an ideal donor

Transfusion starts with selection of an ideal donor, donor must have undergone routine physical and hematological evaluation, healthy with normal PCV, blood typed should have completed vaccinations and deworming and also free from any blood parasite and infectious diseases (Sharma et al., 2009, Battaglia and Steele, 2020, Ramakant et al., 2023).

Compatibility testing

Compatibility testing is a serologic method used to detect incompatibilities between donor and recipient blood. It screens for a general reaction between a specific donor and recipient and can therefore detect antibody reactions to known blood-type antigens, as well as those not tested for by blood typing. Different techniques for compatibility testing are standard laboratory agglutination assay, gel tube assay, immunochromatographic strip assay.

The basis of all current crossmatch testing involves mixing recipient and donor samples and screening for antibodies binding to RBC surface antigens. The compatibility testing is generally done in 2 parts: a major and minor crossmatch (Kristin, 2009, Zaremba et al., 2019).

Standard Laboratory Agglutination Assay

The conventional crossmatch technique involves separating serum and RBCs from donor and recipient blood samples, followed by preparation of washed RBC suspensions. Donor and recipient samples are mixed in major crossmatch (2 drops of patient serum and 2 drops of donor RBC suspension) and minor crossmatch (2 drops of patient RBC suspension and 2 drops of donor serum) combinations along with Control with 1 drop of patient RBC suspension and 2 drops of patient serum and incubated before evaluation for agglutination or hemolysis. The presence of either reaction indicates incompatibility between donor and recipient blood (Zaremba et al., 2019).

Gel Tube Assay

The gel tube method uses microtubes prefilled with dextran–acrylamide gel to detect agglutination reactions. Washed RBCs and serum are added to the gel column

and centrifuged after incubation. Compatible RBCs migrate to the bottom of the tube, whereas agglutinated cells remain trapped within the gel matrix. The degree of agglutination is graded on a scale from 0 to 4+, with higher grades indicating incompatibility (Zaremba et al., 2019).

Immunochromatographic Strip Assay

The immunochromatographic strip assay is a rapid lateral flow test used for crossmatching. Washed RBCs and plasma are mixed with buffer and allowed to migrate along a strip coated with anti-canine antiglobulins. If immunoglobulins or complement components are bound to RBCs, they are captured at the detector line, producing a visible red band that indicates incompatibility. This method reduces false positive reactions caused by autoagglutination (Zaremba et al., 2019).

Adverse transfusion reactions

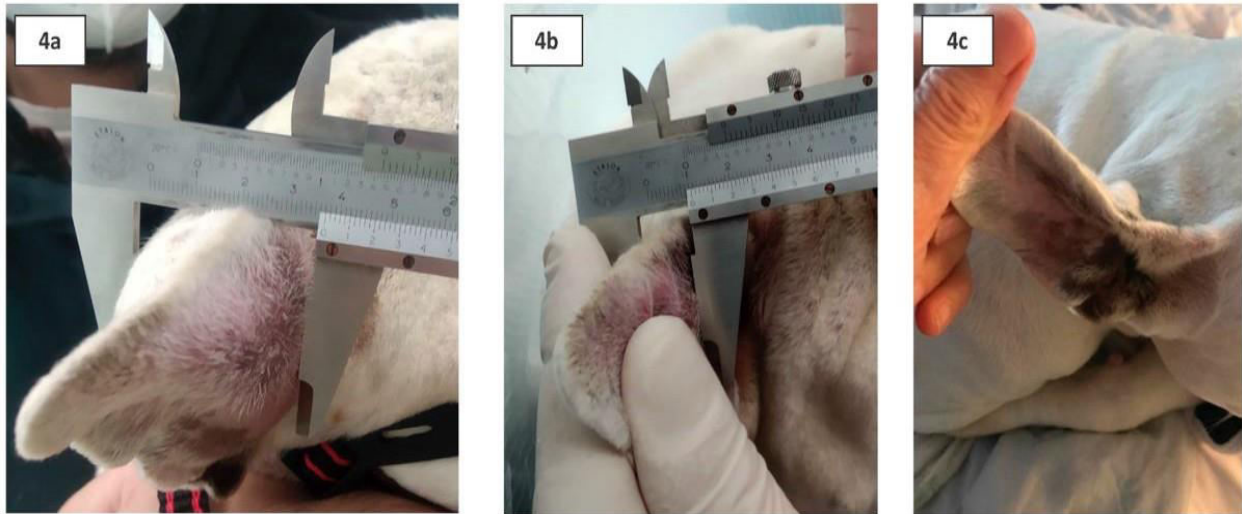
Transfusions are without risk, although potentially lifesaving, they carry substantial risk for development of adverse transfusion reactions (Blumberg et al., 2006).

Adverse transfusion reactions are unintended responses occurring in a patient following transfusion of blood or blood components. These reactions are broadly classified into immunologic and non-immunologic types. Immunologic reactions result from antigen– antibody interactions between donor and recipient blood components, whereas non- immunologic reactions arise from physical or chemical alterations in blood products, contamination, or volume overload. Based on the time of onset, reactions are further categorized as acute (occurring within 24 hours of transfusion) or delayed (occurring after 24 hours) (Davidow et al., 2021; Ramakant et al., 2023).

Several transfusion reactions have been reported in dogs, including febrile non-hemolytic transfusion reactions (FNHTR), allergic reactions, acute hemolytic transfusion reactions, transfusion-associated circulatory overload (TACO), and transfusion-related acute lung injury (TRALI).

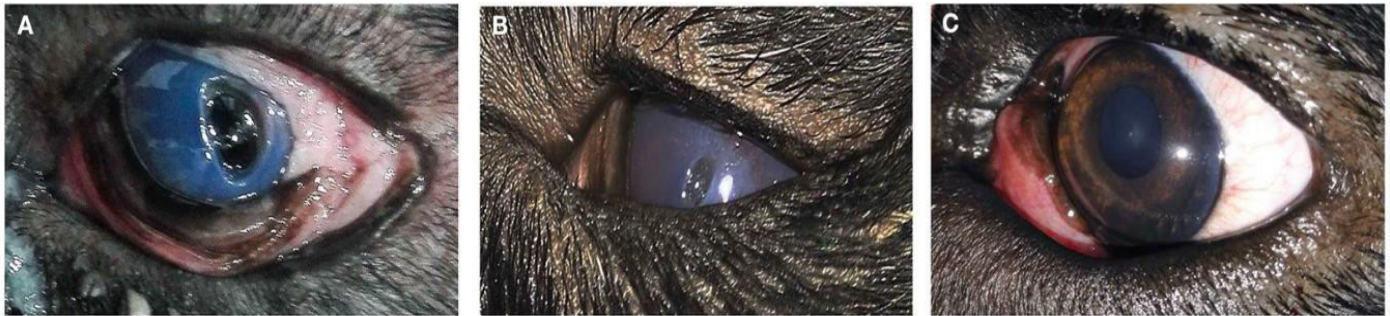
Among these, FNHTR is the most commonly reported reaction (53%), followed by allergic reactions (15%), acute hemolytic transfusion reactions (14%), TACO (10%), and TRALI (8%) (Davidow et al., 2021; Weinstein, 2022).

Figure.1 Pictures of canine aural hematoma. The ear thickness was measured with a caliper before (letter a) and after (letters b and c) the PRP treatment. The swelling was decreased, and the ear came back to its natural thickness without scars.



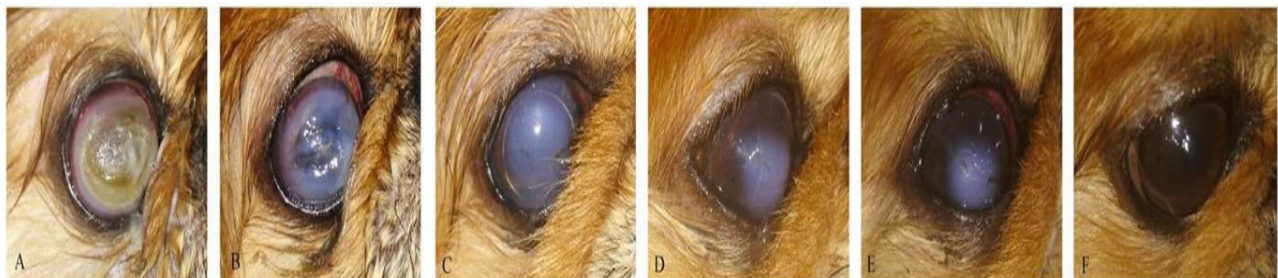
Source: [Palagiano et al., 2023](#)

Figure.2 A photography series of Rottweiler dog showing (A) deep ulcer with keratitis (B) reduction of ulcer size after 2-week post-injection (C) complete healing of ulcer after 3 months of injection.



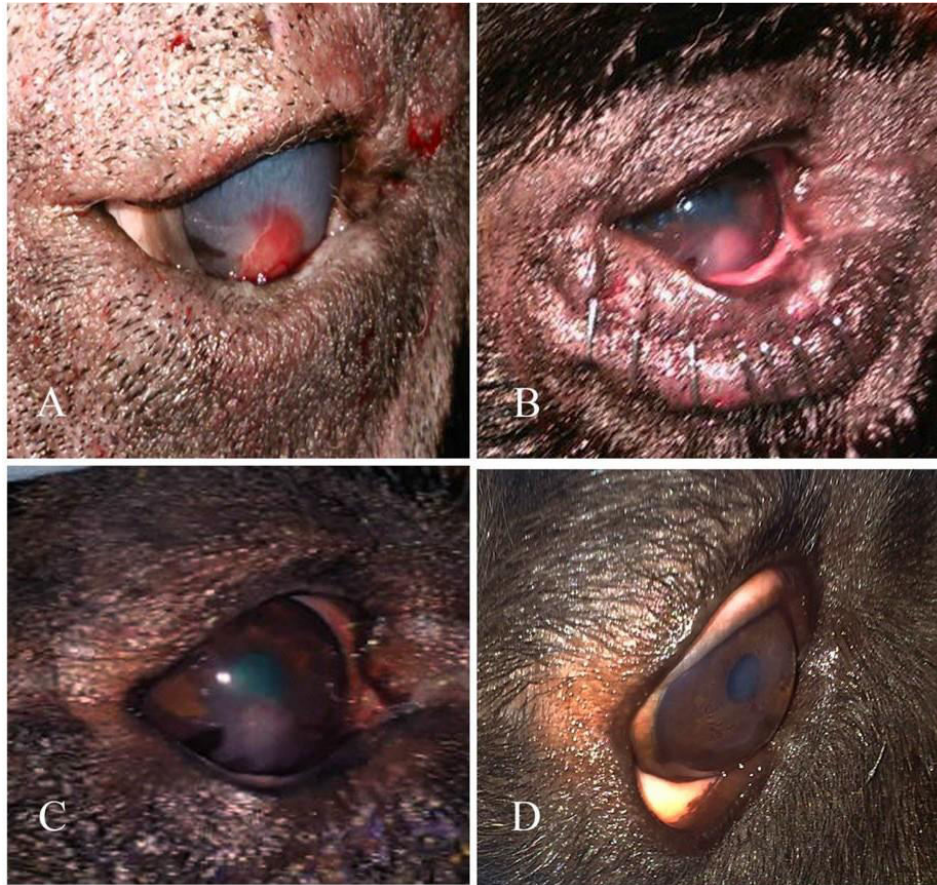
Source: [Farghali et al., 2021](#)

Figure.3 A photography series of Pekingese dog showing (A) melting ulceractive keratitis (B–F) gradual healing and reduction of keratitis after 2-week post-injection till complete healing at 3 months.



Source: [Farghali et al., 2021](#)

Figure.4 A photography series of Rottweiler dog showing (A) entropion with corneal ulcer (B) post-operative after surgical correction of entropion and injection of PRP (C) reduction of corneal ulcer size after 1-week post-injection (D) complete healing of ulcer after 2 weeks of injection.



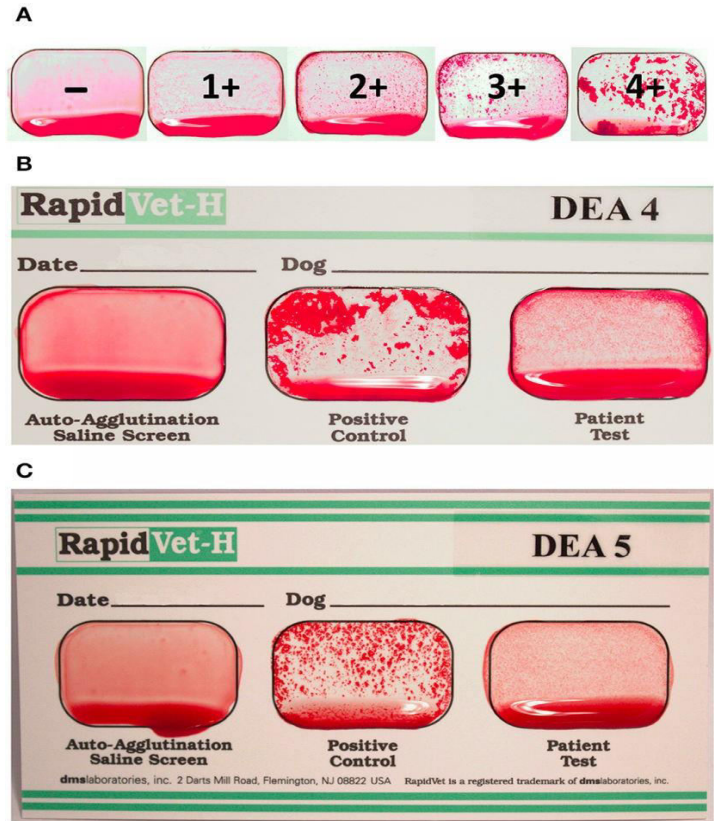
Source: [Farghali et al., 2021](#)

Figure.5 (a): intra-articular injection of liquid PRP in a stifle joint to treat osteoarthritis. (b): Topical application of PRP in a chronic difficult-to-heal wound over the point of the shoulder. (c): Application of PRP for wound bed in gel formulation in a chronic difficult-to-heal wound over the point of the shoulder.



Source: [Perinelli et al., 2020](#)

Figure.6 New agglutination cards for DEA 4 and DEA 5 typing of dogs. (A) Grading of the agglutination reaction strength from – to 4+. (B) DEA 4+ and (C) DEA 5+ showing weak agglutination reactions (1+).



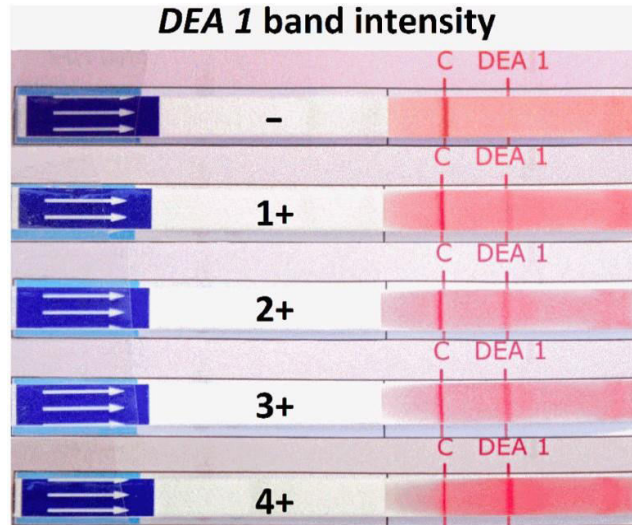
Source: [Ebelt et al., 2020](#)

Figure.7 Gel column typing results for Dal, Kai 1, Kai 2, DEA 4, and DEA 5 from one dog. The blood sample from this dog shows a typical typing pattern: 4+ agglutination reactions for Dal and Kai 1, no agglutination for Kai 2, a 3+ agglutination reaction for DEA 4, and no agglutination for DEA 5. Red blood cells at the top (4+) to within the gel mean positive agglutination reactions.



Source: [Ebelt et al., 2020](#)

Figure.8 The varied binding intensities to a monoclonal anti-DEA 1 antibody at the DEA 1 position on the strip were graded from – (no band, negative) to 1+ to 4+ (band, positive). Red blood cells in suspension migrated in the membrane; the C (control) band had to show for it to be a valid test.



Source: *Ebelt et al., 2020*

Table.1 Major Blood Components Used in Veterinary Transfusion Medicine

Component	Definition	Composition	Indications	Storage Conditions	References
Packed Red Blood Cells (pRBCs)	Concentrated erythrocytes obtained after centrifugation of whole blood with removal of most plasma	Predominantly RBCs with minimal plasma; small quantity of leukocytes may remain	Treatment of anemia caused by hemorrhage, hemolysis, or ineffective erythropoiesis	20 days in CPDA-1 or up to 35 days in CPDA-1 with additive solution (Optisol) at 1–6 °C	<i>Jha et al., (2013); Callan, 2022; Guillaumin and Kofron, 2023</i>
Leukoreduced RBCs	Packed RBCs processed through leukoreduction filters to remove leukocytes prior to storage	RBCs with most leukocytes removed	Management of anemia; reduces transfusion reactions such as non-hemolytic febrile reactions and immunomodulation	Up to 37 days in CPDA at 1–6 °C	<i>Davidow, 2013; Ramakant et al., (2023)</i>
Platelet-Rich Plasma (PRP)	Plasma fraction with supraphysiological platelet concentration obtained through double centrifugation of whole blood	Platelets and plasma enriched with growth factors including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-beta1 (TGF-B1), Epidermal (EGF), basic fibroblast growth factor (b-FGF), and hepatocyte growth factor(HGF)	Severe thrombocytopenia or thrombocytopenia with hemorrhage; also used in wound healing, tissue regeneration, orthopedic and ophthalmic conditions	Typically prepared fresh and used immediately or stored short-term under controlled conditions	<i>Baranidharan et al., (2018); Alves et al., (2021); Farghali et al., (2021); Palagiano et al., (2023); Sharun et al., (2023); Qiu et al., (2024)</i>
Platelet Concentrate	Highly concentrated platelet product obtained by additional of PRP or through plateletpheresis	Concentrated platelets suspended in small volume of plasma	Severe thrombocytopenia or hia with active hemorrhage or prior to invasive procedures	Up to 5 days at 22 °C in gas-permeable bags with Constant agitation	<i>Davidow, 2013; Hakami et al., (2024)</i>

Lyophilized Platelets	Platelets preserved through stabilization and freeze-drying (lyophilization) to extend shelf life	Stabilized platelets prepared by aldehyde cross-linking followed by lyophilization	Severe thrombocytopenia or thrombocytopenia with active hemorrhage or before invasive procedures	Up to 2 years at -20 to -30°C	Davidow, 2013; Kim and Han, 2023
Fresh Frozen Plasma (FFP)	Plasma separated from whole blood and frozen within 8 hours of collection to preserve coagulation factors	Contains coagulation factors, anticoagulants (antithrombin, α2-macroglobulin), and albumin	Treatment of coagulation disorders, hypoproteinemia, burns, and prophylactic use before invasive procedures	Up to 1 year at -20 to -30 °C	Pai <i>et al.</i> , (2023); Guillaumin and Kofron, 2023
Cryoprecipitate	Cold-insoluble protein fraction obtained after slow thawing and centrifugation of FFP	Rich in von Willebrand factor, Factor VIII, Factor XIII, and fibrinogen	Treatment of Hemophilia A, von Willebrand disease, and hypofibrinogenemia	Up to 10 months at -20 to -30 °C	Henna and Curry, 2016; Prittie, 2021; Lam, 2023
Cryoprecipitate-Poor Plasma	Plasma supernatant remaining after removal of cryoprecipitate	Contains stable Coagulation factors II, VII, IX, and X along with Anticoagulant and Fibrinolytic proteins and albumin	Coagulation factors II, VII, IX, or X associated with active hemorrhage when vWF replacement is not required	Up to 1 year at -20 to -30 °C	Drinkhouse <i>et al.</i> , (2018); Davidow, 2013

Table.2 Major and Minor crossmatching

Type	Donor's	Recipient's
Major Crossmatch	Red cells	Serum
Minor Crossmatch	Serum	Red Cells

Febrile Non-Hemolytic Transfusion Reaction (FNHTR)

FNHTR is characterized by a body temperature exceeding 39°C or an increase of >1°C from baseline during or within 4 hours after transfusion. These reactions may result from recipient antibodies reacting with donor leukocytes or platelets, or from pro-inflammatory cytokines accumulated in stored blood products. Most cases are mild and self-limiting (Fung and Heedle, 2013; Davidow *et al.*, 2021; Blois, 2016).

Allergic Transfusion Reaction

Allergic transfusion reactions are acute immunologic reactions mediated by type I hypersensitivity to plasma proteins or other antigens present in donor blood products. Clinical signs may include urticaria, erythema, pruritus, facial swelling, gastrointestinal disturbances, and in severe cases anaphylaxis. These reactions typically occur during or within 4 hours of transfusion (Davidow *et al.*, 2021).

Acute Hemolytic Transfusion Reaction (AHTR)

Acute hemolytic transfusion reactions occur due to immune-mediated destruction of transfused RBCs, usually resulting from blood type incompatibility between donor and recipient. Antibodies, primarily IgM or IgG, bind to donor RBC antigens and activate the complement cascade, leading to intravascular hemolysis. Clinical signs may include fever, tachycardia, vomiting, hemoglobinuria, dyspnea, hypotension, and signs of shock (Zaremba *et al.*, 2019; Davidow *et al.*, 2021; Massey *et al.*, 2023).

Transfusion-Associated Circulatory Overload (TACO)

TACO is a non-immunologic reaction caused by rapid or excessive transfusion leading to increased intravascular volume and hydrostatic pulmonary edema. It typically occurs during or within 6 hours of transfusion and presents with respiratory distress, tachypnea, and signs of pulmonary edema (Roubinian and Murphy, 2015; Davidow *et al.*, 2021; Bulle *et al.*, 2022).

Transfusion-Related Acute Lung Injury (TRALI)

TRALI is an acute immunologic complication caused by antigen–antibody interactions that activate neutrophils in pulmonary capillaries, leading to endothelial damage and non- cardiogenic pulmonary edema. It usually develops during or within 6 hours of transfusion and is characterized by acute hypoxemia and pulmonary infiltrates on thoracic radiographs (Roubinian and Murphy, 2015; Davidow *et al.*, 2021).

Prevention and management of adverse transfusion reactions

Prevention of adverse transfusion reactions: To prevent transfusion reactions, it is essential to follow a series of precautionary measures. The selection of an ideal donor and thorough compatibility testing are critical step. Continuous and careful monitoring of the recipient throughout the transfusion process is equally important. If any adverse reactions are observed, the transfusion must be stopped immediately (Dadke and Galdhar, 2021, Choudhary *et al.*, 2017).

Management of adverse transfusion reaction: In cases of allergic or anaphylactic reactions, epinephrine should be administered intramuscularly at a dose of 0.1–0.2 mg/kg. For managing TACO, furosemide is given intravenously at 1–2 mg/kg. Additionally, antihistamines like chlorpheniramine at the dose of 0.5 mg/kg IM and corticosteroids such as dexamethasone at the dose of 0.5–1 mg/kg SC may be used depending on the clinical situation (Dadke and Galdhar, 2021, Davidow *et al.*, 2021)

Current initiatives

The demand for animal blood is fast-growing through the years as there is increased pet adoption. The enormous advancement in the veterinary field has seen researchers developing blood banks for pets (Aravindh and Jacob, 2021).

Blood Banks: It helps to ease the usage of blood in the recipients and avoids several cross- reaction. It provides convenient access to blood and relieves the suffering of the animals. It gives immediate action in finding a correct match for the animal without any further delay. The first canine to canine blood transfusion was done as early as the year 1665. Since then, whole blood and blood products have been used to treat many diseases

and for surgery in veterinary medicine. In the year 2007, a successful blood bank was established in the United Kingdom. In India also, it has gained importance at the local, regional and national levels due to the need for blood during various emergencies. India’s first blood bank for dogs was started by the Tamil Nadu University of Veterinary and Animal Sciences (TANUVAS). Another blood bank was initiated by the Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) in Ludhiana in 2020 (Aravindh and Jacob, 2021).

In conclusion, Transfusion medicine in canine practice serves as a critical and life-saving modality in emergency situations, particularly for critically ill patients. The advent of modern component therapy has enabled more targeted treatments, thereby improving efficiency and patient outcomes. Successful transfusion relies heavily on accurate blood typing, compatibility testing and careful donor selection. Component transfusion enabled targeted and efficient clinical management of various hematological and systemic disorders. It can be a preferred choice in emergencies with specific requirements. Additionally, the use of platelet-rich plasma in veterinary ophthalmology is an emerging area with promising implications. Recent advancements, including the establishment of veterinary blood banks and mobile donation units, have improved accessibility and availability of safe blood products, reflecting the growing importance of transfusion medicine in modern veterinary care.

Acknowledgments

Mimansha Sahu: Conceptualization, primary drafting and overall manuscript development; S. K. Maiti, S. L. Ali, Nidhi Rawat, Nitin Gade and B.P.S. Kanwar: Conceptualization, supervision, critical review; Prafulla Kashyap and Manish Kumar Baghel: Preparation of tables and technical support; Samidha Manikpuri, Priyanka Singh, Deeksha Sahu and Bhumika Kaushik: Literature collection, compilation and editing.

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curation, supervision, writing—reviewing the final version of the manuscript. Manish Kumar Baghel: Investigation, formal analysis, writing—original draft. Samidha Manikpuri: Validation, methodology, writing—reviewing. Priyanka Singh:—Formal analysis, writing—review and editing. Deeksha Sahu: Investigation, writing—reviewing. Bhumika Kaushik: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Mimansha Sahu, Maiti S. K., Ali S. L., Nidhi Rawat, Nitin Gade, Kanwar B. P. S., Prafulla Kashyap, Manish Kumar Baghel, Samidha Manikpuri, Priyanka Singh, Deeksha Sahu and Bhumika Kaushik. 2026. Recent Advances in Canine Blood Transfusion: A Review. *Int.J.Curr.Microbiol.App.Sci*. 15(6): 70-81.

doi: <https://doi.org/10.20546/ijcmas.2026.1506.007>